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Associations of maternal and fetal vitamin D status with childhood body composition and cardiovascular risk factors

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Abstract

Maternal vitamin D deficiency during pregnancy may have persistent adverse effects on childhood growth and development. We examined whether 25-hydroxyvitamin D (25(OH)D) concentrations during pregnancy and at cord blood were associated with childhood body composition and cardiovascular outcomes. This study was embedded in a population-based prospective cohort in Rotterdam, The Netherlands, among 4,903 mothers and their offspring. We measured 25(OH)D concentrations at a median gestational age of 20.4 weeks (95% range 18.5–23.4 weeks) and at birth (40.1 weeks [95% range 35.8–42.3 weeks]). 25(OH)D concentrations were categorized into severely deficient (<25.0 nmol/L); deficient (25.0 to 49.9 nmol/L); sufficient (50.0 to 74.9 nmol/L) and optimal (≥75.0 nmol/L). At 6 years, we measured childhood body mass index; fat and lean mass by Dual-energy X-ray Absorptiometry; blood pressure; and serum cholesterol, triglycerides, and insulin concentrations. Compared with children from mothers with optimal 25(OH)D concentrations (≥75.0 nmol/L), those of severely deficient vitamin D (<25.0 nmol/L) mothers had a 0.12 standard deviation score (SDS); (95% Confidence Interval (CI) [0.03, 0.21]) higher fat mass percentage and a 0.13 SDS (95% CI [−0.22, −0.04]) lower lean mass percentage. These associations remained after adjustment for current child vitamin D status. Maternal and cord blood 25(OH)D concentrations were not associated with cardiovascular risk factors in childhood. In conclusion, severe maternal 25(OH)D deficiency (<25.0 nmol/L) during pregnancy is associated with an adverse childhood body composition profile, but we did not observe evidence for an association with childhood cardiovascular risk factors. Further studies are needed to replicate our findings, to examine the underlying mechanisms, the causality of the associations, and the potential for public health interventions.

KEYWORDS

adiposity, body composition, cardiovascular risk factors, pediatrics, pregnancy, vitamin D

1 | INTRODUCTION

Vitamin D is essential for fetal development because of its significant role during proliferation, differentiation, and maturation processes of cells, including adipose tissues and muscle cells (Brown, Dusso, & Slatopolsky, 1999; Gale et al., 2008; Pasco et al., 2008; Wood, 2008). We have previously shown that suboptimal concentrations of maternal vitamin D are associated with low birth weight and small size for gestational age (Miliku et al., 2016), which are known to be associated with cardiovascular risk factors in the offspring (Horikoshi et al., 2013; Lawlor et al., 2007). Studies in adults suggest that vitamin D plays an important role in cardiovascular protection and body composition profile (Vitezova et al., 2016; T. J. Wang et al., 2008; Wimalawansa, 2016). Furthermore, cross-sectional studies in children have reported associations of lower vitamin D status with higher adiposity measures and cardiovascular risk factors such as blood pressure, plasma lipids, and insulin concentrations (Gilbert-Diamond et al., 2010; Petersen et al., 2015; Williams et al., 2012). The relations of vitamin D with calcium are well known. It has previously been reported that calcium supplementation during pregnancy has long-term effects on childhood blood pressure (Belizan et al., 1997). Thus far, only few studies have explored the associations of circulating fetal 25(OH)D concentrations with later childhood adiposity, body composition, and cardiovascular risk factors, with inconsistent results (Crozier et al., 2012; Gale et al., 2008; Krishnaveni et al., 2011; Rytter et al., 2016; Williams et al., 2013). These inconsistent findings may be due to differences between study populations. The small sample sizes of the previous studies could have limited their ability to detect associations (Crozier et al., 2012; Gale et al., 2008; Krishnaveni et al., 2011).

Therefore, for the current study, we hypothesized that adverse exposure to suboptimal 25(OH)D concentrations during critical periods of fetal organ development affects childhood adiposity and cardiovascular health. In a population-based prospective cohort study among 4,903 mother and children pairs, we explored the associations of 25(OH)D concentrations during midpregnancy and in cord blood with body composition and cardiovascular risk factors at school-age. We also explored whether any association was explained by child's current 25(OH)D concentrations.

Key messages

- Severely deficient maternal vitamin D concentrations (<25.0 nmol/L) during midpregnancy are associated with higher fat mass and lower lean mass at school-age.
- Maternal and cord blood vitamin D concentrations are not associated with childhood cardiovascular outcomes.
- The associations of maternal vitamin D concentrations and childhood body composition remained similar after adjustment for child current vitamin D concentrations.
- These results are important from an etiological perspective suggesting that suboptimal vitamin D concentrations in pregnant women may have persistent effects on their offspring body composition.

2 | METHODS

2.1 | Design and study population

This study was embedded in the Generation R Study, a population-based prospective cohort study from fetal life onwards in Rotterdam, The Netherlands (Kruithof et al., 2014). All children were born between April 2002 and January 2006. Enrolment in the study was aimed at early pregnancy but was possible until the first month after delivery. The study protocol was approved by the local Medical Ethical Committee. Written consent was obtained for all children. Second trimester 25(OH)D concentrations were measured in 7,176 mothers. For the present study, we excluded pregnancies leading to twin births (n = 78) and children with congenital heart abnormalities (n = 21). Among 7,077 singletons available for follow-up measurements, 2,174 did not visit the research centre at the age of 6 years. Thus, the cohort for analysis composed of 4,903 mothers and children with vitamin D measurements and childhood outcomes available. (Figure S1).

2.2 | Maternal and cord 25(OH)D blood concentrations

As previously described, maternal venous blood samples were collected in second trimester (median 20.4 weeks of gestation, 95% range 18.5–23.4 weeks), whereas umbilical cord blood samples were collected at birth (median 40.1 weeks of gestation, 95% range 35.8–42.3 weeks; Kruithof et al., 2014). Plasma levels of 25(OH)D_2 and 25(OH)D_3 was quantified using isotope dilution liquid chromatography-tandem mass spectrometry at the Queensland Brain Institute (Brisbane, Australia) approved by vitamin D External Quality Assessment Scheme. Assay accuracy was assessed at four concentration levels for 25(OH)D_3 (48.3, 49.4, 76.4, and 139.2 nmol/L) and a single level for 25(OH)D_2 (32.3 nmol/L) and was excellent at all concentration levels tested (<10% and <17%, respectively); (Miliku et al., 2016; Vinkhuyzen et al., 2015). Total 25(OH)D was calculated as the sum of 25(OH)D_2 and 25(OH)D_3 measured in plasma (Eyles et al., 2009). According to current recommendations, we categorized vitamin D status into severely deficient: <25.0 nmol/L (<10.0 mg/L); deficient: 25.0 to 49.9 nmol/L (10.0 to 19.9 mg/L); sufficient: 50.0 to 74.9 nmol/L (20.0 to 29.9 mg/L); optimal ≥75.0 nmol/L (≥30.0 mg/L); (Holick, 2007; Miliku et al., 2016; Ross et al., 2011).

2.3 | Body composition and cardiovascular outcomes

Children's anthropometrics and body composition were measured at a median age of 6.0 years (95% range 5.7–8.0) by well-trained staff in our research centre (Jaddoe et al., 2012). Height (m) was determined in standing position to the nearest millimetre without shoes using a Harpenden stadiometer (Holtain Limited, Dyfed, UK). Weight (kg) was measured using a mechanical personal scale (SECA, Almere, The Netherlands) and body mass index (BMI; kg/m^2), and BMI-for-age z-

scores according to WHO guidelines were calculated. We performed whole body Dual-energy X-ray Absorptiometry (DXA) scans (iDXA, GE-Lunar, 2008, Madison, WI, USA) that analysed fat and lean mass. We calculated fat mass percentage as (fat mass [kg]/weight [kg]), fat mass index as (fat mass [kg]/height $[m]^2$), and lean mass percentage as (lean mass [kg]/weight [kg]).

Blood pressure was measured at the right brachial artery four times with 1-min intervals, using the validated automatic sphygmanometer Datascope Accutor Plus (Paramus, NJ, USA; Wong, Tz Sung, & Leung, 2006). We calculated the mean value for systolic and diastolic blood pressure using the last three blood pressure measurements of each participant. Nonfasting blood samples were collected to measure total cholesterol, triglycerides, and insulin concentrations, using Cobas 8000 analyser (Roche, Almere, The Netherlands). Quality control samples demonstrated intra and inter-assay coefficients of variation ranging from 0.77% to 1.17% and 0.87% to 1.69%, respectively.

2.4 | Covariates

We used questionnaires at enrolment in the study (median 13.5 weeks of gestation) to collect information about maternal age; ethnicity; educational level; parity; and on smoking, folic acid, and vitamins supplementation during pregnancy (Jaddoe et al., 2012). Maternal energy and calcium dietary intake during pregnancy were measured at enrolment with a validated semiquantitative food frequency questionnaire (Klipstein-Grobusch et al., 1998). Ethnicity and educational level were defined according to the classification of Statistics Netherlands. Higher education was defined as having at least some higher/university education. Maternal height and weight were self-reported and prepregnancy BMI was calculated (kg/m^2). The date of blood sampling was categorized into spring, summer, fall, and winter based on the Dutch standard seasons. Infant sex, gestational age, and weight at birth were obtained from midwives, medical records, and hospital registries. Information on breastfeeding was collected using postnatal questionnaires (Miliku et al., 2015). At the age of 6 years, child participation at sports was reported and 25(OH)D concentrations were measured in a subgroup of 3,068 subjects as described in detail elsewhere (Voortman et al., 2015).

2.5 | Statistical methods

We performed a nonresponse analysis by comparing subject characteristics between children with and without follow-up body composition and cardiovascular outcomes using *t* tests, chi-square tests, and Mann–Whitney tests. We created standard deviations scores (SDS) for all outcomes to enable comparison between effect estimates. We used multivariable linear regression models to assess the associations of maternal and cord blood 25(OH)D concentrations with childhood BMI; fat mass percentage; lean mass percentage; systolic and diastolic blood pressure; and total-cholesterol, triglycerides, and insulin concentrations. We log-transformed the not-normally distributed outcomes, childhood triglycerides, and insulin concentrations. Vitamin D concentrations were analysed both continuously per standard deviation increase and using clinical cut-offs (Holick, 2007; Miliku et al., 2016; Ross et al., 2011). The regression models were first adjusted for child's sex, child's age at outcome measurements and maternal ethnicity (basic models); subsequently additionally for maternal age, education, prepregnancy BMI, parity, smoking, folic acid and vitamins supplement use, energy and dietary calcium intake during pregnancy, season when blood samples were drawn; and for child's birth weight, gestational age at birth, breastfeeding in early life and playing sports at the age of 6 years (adjusted model). The models on cardiovascular outcomes were additionally adjusted for childhood BMI (adjusted model). The covariates were included in the models based on their associations with adiposity and cardiovascular outcomes in previous studies (Taylor, Gold, Manning, & Goulding, 1997; Timmermans et al., 2008; Williams et al., 2013), or a change in effect estimates of >10%. To explore if childhood 25(OH)D status explained the associations between maternal 25(OH)D and childhood body composition measures, we performed a sensitivity analysis in which we additionally adjusted for child 25(OH)D in the subgroup of n = 3,068 children with data on 25(OH)D concentrations available (childhood vitamin D model). Because of the correlations between different outcomes, we did not apply adjustment for multiple testing in the main analyses. However, as sensitivity analysis, we also present statistical significance after taking account for three groups of outcomes (body composition, blood pressure, and blood concentrations; $P < 0.017$ [0.05/3]). Ethnicity is strongly associated with 25(OH)D concentrations; therefore, we first adjusted the regression models for maternal ethnicity and second, we restricted the analyses to Europeans only, the largest ethnic subgroup in our cohort (Vinkhuyzen et al., 2015). Since the interactions of maternal 25(OH)D with child sex were not significant, we did not stratify our analyses on child sex. To diminish potential bias associated with attrition, missing values of covariates (less than 23.5%) were multiple imputed by generating five independent datasets using the Markov Chain Monte Carlo method. The multiple imputation procedure was based on the correlation between each variable with missing values and the other subject characteristics (Rubin & Schenker, 1991; Sterne et al., 2009). Subjects characteristics before and after imputation are shown in Table S1. Statistical analyses were performed using SPSS version 21.0 (SPSS Inc., Chicago, IL, USA).

3 | RESULTS

3.1 | Subject characteristics

Table 1 shows the subject characteristics. Overall, 8.5% of the mothers had a BMI >30 kg/m^2. The distribution of 25(OH)D concentrations based on different used cut-offs is given in Table S2. Results from the nonresponse analysis showed that as compared with children who did not have follow-up measurements at the age of 6 years, those who did have these measurements had mothers who were on average higher educated and had higher 25(OH)D concentrations during pregnancy (Table S3). The correlation coefficients of the investigated outcome variables are given in Table S4. The correlation coefficient between midpregnancy maternal 25(OH)D concentrations and cord blood 25(OH)D concentrations was r = 0.61.

TABLE 1 Subject characteristics (N = 4,903)

Maternal characteristics	
Age (years)	30.4 (5.03)
Prepregnancy body mass index (kg/m^2)	22.7 (18.1, 34.8)
<18.5	3.8
18.5–24.9	67.8
25–29.9	19.9
30 or higher	8.5
Nulliparous (%)	57.8
Higher education (%)	45.1
Ethnicity (%)	
European	60.6
Cape Verdean	4.5
Dutch Antillean	2.7
Moroccan	5.9
Turkish	8.9
Surinamese	8.2
Other	9.2
Smoking during pregnancy (%)	
Never	74.2
Until pregnancy was known	9.4
Continued	16.4
Multivitamin supplement use (%)	31.1
Folic acid supplement use (%)	
No	26.8
Start in the first 10 weeks	31.6
Start periconceptional	41.6
Energy intake (kcal)	2,049 (490)
Dietary calcium intake (mg)	1,106 (329)
Blood concentrations of 25(OH)D (nmol/L)	50.4 (7.5, 122.5)
Season when blood sample was taken (%)	
Spring	28.6
Summer	22.6
Autumn	25.1
Winter	23.7
Infant characteristics	
Girls (%)	50.4
Gestational age at birth (week)	40.1 (35.8, 42.3)
Birth weight (g)	3,431 (554)
Ever breastfed in the first 4 months (%)	92.3
25(OH)D concentration in cord blood at birth (nmol/L)	28.8 (5.0, 81.7)
Season when cord blood sample was taken (%)	
Spring	27.6
Summer	26.6
Autumn	22.5
Winter	23.3
Child characteristics at 6 years' visit	
Age (years)	6.0 (5.7, 7.8)
Height (cm)	119.4 (6.0)
Weight (kg)	22.6 (17.6, 33.8)
Playing sports, yes (%)	43.3
Childhood blood concentrations of 25(OH)D (nmol/L)	64.0 (18.0, 133.0)

(Continues)

TABLE 1 (Continued)

Body composition	
Body mass index (kg/m^2)	15.9 (13.6, 21.2)
Body mass index for age z-scores	0.36 (−1.30, 2.74)
Fat mass percentage	24.9 (5.7)
Fat mass index	4.1 (3.7)
Lean mass percentage	71.5 (5.4)
Cardiovascular risk factors	
Systolic blood pressure (mmHg)	102.7 (8.3)
Diastolic blood pressure (mmHg)	60.7 (6.9)
Total-cholesterol (mmol/L)	4.2 (0.6)
Triglycerides (mmol/L)	0.95 (0.4, 2.3)
Insulin (pmol/L)	114.2 (17.0, 397.7)

Note. 25(OH)D: 25-hydroxyvitamin D.

*Values are percentages for categorical variables, means (*SD*) for continuous variables with a normal distribution, or medians (95% range) for continuous variables with a skewed distribution.

3.2 | Maternal and cord 25(OH)D concentrations and childhood body composition

Table S5 shows the results from the basic regression models for the associations of maternal and cord blood 25(OH)D concentrations and childhood body composition measures. Table 2 shows the associations of maternal and cord blood 25(OH)D concentrations and childhood body composition measures. Maternal or cord blood 25(OH)D were not associated with childhood BMI (Table 2) or BMI for age according to WHO guidelines (Table S6). As compared with children of mothers who had optimal 25(OH)D concentrations (≥75.0 nmol/L), those of mothers who were severely vitamin D deficient (<25.0 nmol/L) had a 0.12 standard deviation score (SDS; 95% confidence interval, CI [0.03, 0.21]) higher fat mass percentage and a 0.13 SDS (95% CI [−0.22, −0.04]) lower lean mass percentage. The associations of maternal 25(OH)D status with childhood fat and lean mass percentage remained significant after considering multiple testing and after additional adjustment for childhood vitamin D status. When we examined the observed associations in the subgroup of children with vitamin D measurements at the age of 6 years, the general

TABLE 2 Associations of maternal 25(OH)D concentrations and body composition measures at the age of 6 years

Fetal 25(OH)D concentrations	**Difference in outcome measure (95% Confidence Interval)**				
Maternal 25(OH)D concentrations	Body mass index (*n* = 4,898)	Fat mass percentage (*n* = 4,805)	Fat mass percentage (*n* = 3,068)	Lean mass percentage (*n* = 4,805)	Lean mass percentage (*n* = 3,068)
	Adjusted model	*Adjusted model*	*Childhood vitamin D model*	*Adjusted model*	*Childhood vitamin D model*
<25.0 nmol/L (*N* = 1,142)	0.06 (−0.04, 0.16)	**0.12 (0.03, 0.21)*** †	0.12 (0.01, 0.24)*	**−0.13 (−0.22, −0.04)*** †	**−0.13 (−0.24, −0.02)***
25.0 to 49.9 nmol/L (*N* = 1,281)	0.03 (−0.05, 0.11)	0.05 (−0.03, 0.12)	0.02 (−0.07, 0.11)	−0.05 (−0.12, 0.03)	−0.02 (−0.11, 0.07)
50.0 to 74.9 nmol/L (*N* = 1,195)	0 (−0.07, 0.07)	0.02 (−0.05, 0.09)	0.01 (−0.08, 0.09)	−0.02 (−0.09, 0.04)	−0.01 (−0.09, 0.08)
≥75.0 nmol/L (*N* = 1,285)	Reference	Reference	Reference	Reference	Reference
Continuously (per SD)	*−0.01 (−0.05, 0.02)*	*−0.03 (−0.06, 0)*	*−0.03 (−0.07, 0.01)*	***0.03 (0, 0.06)****	*0.04 (0, 0.07)*
Cord blood 25(OH)D concentrations	Body mass index (*n* = 3,048)	Fat mass percentage (*n* = 2,998)	Fat mass percentage (*n* = 1,907)	Lean mass percentage (*n* = 2,998)	Lean mass percentage (*n* = 1,907)
	Adjusted model	*Adjusted model*	*Childhood vitamin D model*	*Adjusted model*	*Childhood vitamin D model*
<25.0 nmol/L (*N* = 665)	0.12 (−0.06, 0.29)	0.04 (−0.13, 0.20)	0.13 (−0.07, 0.34)	−0.04 (−0.21, 0.12)	−0.13 (−0.34, 0.07)
25.0 to 49.9 nmol/L (*N* = 818)	0.16 (−0.01, 0.32)	0.04 (−0.11, 0.20)	0.09 (−0.10, 0.28)	−0.04 (−0.20, 0.11)	−0.08 (−0.28, 0.11)
50.0 to 74.9 nmol/L (*N* = 780)	0.03 (−0.14, 0.20)	−0.03 (−0.19, 0.13)	0.03 (−0.17, 0.23)	0.03 (−0.13, 0.19)	−0.03 (−0.23, 0.17)
≥75.0 nmol/L (*N* = 790)	Reference	Reference	Reference	Reference	Reference
Continuously (per SD)	*−0.04 (−0.08, 0.01)*	*−0.03 (−0.07, 0.01)*	*−0.04 (−0.09, 0.01)*	*0.03 (−0.01, 0.07)*	*0.04 (−0.01, 0.09)*

Note. Values are linear regression coefficients (95% Confidence Interval). Adjusted model is adjusted for maternal characteristics (age, ethnicity, body mass index before pregnancy, parity, education, smoking, folic acid and multivitamin supplements, dietary calcium and energy intake during pregnancy, and season of blood sampling) and child characteristics (sex, birthweight, gestational age at birth, breastfeeding, age at measurements, and playing sports at the age of 6 years). Childhood vitamin D model is adjusted model additionally adjusted for child's current 25(OH)D concentrations. Continuously = maternal and cord blood vitamin D concentrations analysed per 1 standard deviation in 25(OH)D. 25(OH)D: 25-hydroxyvitamin D.

*$P < 0.05$.

†Also significant after applying Bonferroni correction ($P < 0.017$).

TABLE 3 Associations of maternal 25(OH)D concentrations during pregnancy with cardiovascular outcomes at the age of 6 years (*N* = 4,903)

Fetal 25(OH)D concentrations	Difference in outcome measure SDS (95% Confidence Interval)				
Maternal 25(OH)D concentrations	Systolic blood pressure (*n* = 4,550)	Diastolic blood pressure (*n* = 4,550)	Total cholesterol (*n* = 3,281)	Triglycerides (*n* = 3,270)	Insulin (*n* = 3,256)
<25.0 nmol/L (*N* = 1,142)	−0.01 (−0.12, 0.10)	−0.01 (−0.12, 0.10)	0.02 (−0.11, 0.15)	−0.01 (−0.15, 0.12)	−0.06 (−0.20, 0.07)
25.0 to 49.9 nmol/L (*N* = 1,281)	0.01 (−0.08, 0.09)	0.02 (−0.06, 0.11)	0.02 (−0.08, 0.13)	0.01 (−0.09, 0.12)	−0.01 (−0.12, 0.09)
50.0 to 74.9 nmol/L (*N* = 1,195)	−0.01 (−0.09, 0.07)	−0.02 (−0.10, 0.07)	0.07 (−0.03, 0.17)	0.03 (−0.06, 0.13)	−0.10 (−0.20, 0)
≥75.0 nmol/L (*N* = 1,285)	Reference	Reference	Reference	Reference	Reference
Continuously (per *SD*)	0.01 (−0.03, 0.05)	0 (−0.04, 0.04)	0 (−0.04, 0.05)	0 (−0.05, 0.04)	0.02 (−0.03, 0.06)
Cord blood 25(OH)D concentrations	Systolic blood pressure (*n* = 2,847)	Diastolic blood pressure (*n* = 2,847)	Total cholesterol (*n* = 2,042)	Triglycerides (*n* = 2,038)	Insulin (*n* = 2,201)
<25.0 nmol/L (*N* = 665)	−0.07 (−0.26, 0.13)	−0.07 (−0.27, 0.13)	0.07 (−0.17, 0.32)	0.09 (−0.15, 0.34)	−0.03 (−0.26, 0.21)
25.0 to 49.9 nmol/L (*N* = 818)	0.02 (−0.20, 0.17)	−0.07 (−0.26, 0.12)	0.19 (−0.03, 0.42)	0.09 (−0.14, 0.32)	0.06 (−0.17, 0.28)
50.0 to 74.9 nmol/L (*N* = 780)	0.02 (−0.17, 0.21)	−0.03 (−0.22, 0.17)	0.10 (−0.14, 0.34)	0.09 (−0.15, 0.33)	0.08 (−0.16, 0.312)
≥75.0 nmol/L (*N* = 790)	Reference	Reference	Reference	Reference	Reference
Continuously (per *SD*)	0.02 (−0.03, 0.07)	0.01 (−0.04, 0.06)	0 (−0.06, 0.05)	−0.01 (−0.07, 0.05)	0.03 (−0.03, 0.09)

Note. Values are linear regression coefficients (95% confidence interval). Adjusted model is adjusted for maternal characteristics (age, ethnicity, body mass index before pregnancy, parity, education, smoking, folic acid and multivitamin supplements, dietary calcium and energy intake during pregnancy, and season of blood sampling) and child characteristics (sex, birthweight, gestational age at birth, breastfeeding, age at measurements, body mass index, and playing sports at the age of 6 years). Continuously = maternal and cord blood vitamin D concentrations analysed per 1 standard deviation in 25(OH)D. 25(OH)D: 25-hydroxyvitamin D.

*$P < 0.05$.

pattern of findings persisted. Children of mothers who were severely vitamin D deficient (<25 nmol/L) had a 0.12 SDS (95% CI [0.01, 0.24]) higher fat mass percentage and a 0.13 SDS (95% CI [−0.24, −0.02]) lower lean mass percentage, as compared with children of mothers who had optimal 25(OH)D concentrations (≥75.0 nmol/L). Cord blood 25(OH)D concentrations were not associated with child body composition. Similar results to fat mass percentage were observed for fat mass index (Table S6). We observed tendencies for similar effect estimates when we restricted the analyses to Europeans only (*N* = 2,974), although the observed associations on fat mass percentage and lean mass percentage did not reached statistical significance due to smaller sample sizes (Table S7).

3.3 | Maternal and cord 25(OH)D concentrations and childhood cardiovascular outcomes

Table 3 shows that maternal and cord blood 25(OH)D concentrations were not associated with childhood blood pressure, total cholesterol, triglycerides, or insulin concentrations. The results from the basic models and the analysis restricted to Europeans only (*N* = 2,974) are given in Tables S8 and S9, respectively, and showed similar results.

4 | DISCUSSION

In this population-based prospective cohort study, we observed that severely deficient maternal 25(OH)D concentrations (<25 nmol/L) during midpregnancy, but not at birth, tended to be associated with higher fat mass and lower lean mass percentage at school-age. These associations remained similar after adjustment for childhood 25(OH)D concentrations. Maternal and cord blood 25(OH)D concentrations were not associated with childhood cardiovascular outcomes.

4.1 | Interpretation and comparison with previous studies

An accumulating body of evidence suggests that suboptimal vitamin D levels are common and related with the risk of cardiovascular disease (Martini & Wood, 2006; L. Wang et al., 2012). Adults studies suggest that low 25(OH)D concentrations are associated with higher BMI and higher fat mass percentage (Jackson et al., 2016; Vitezova et al., 2016). In line with these results, studies in animals show that vitamin D influences development and differentiation of adipocytes and muscle cells (Pasco et al., 2008; Wood, 2008). Also, previous childhood studies suggest a potential role of 25(OH)D concentrations on body composition and cardiovascular outcomes (Gilbert-Diamond et al., 2010; Petersen et al., 2015; Williams et al., 2012). Vitamin D is reported to be inversely associated with the development of adiposity in school-aged children (Gilbert-Diamond et al., 2010). Moreover, childhood vitamin D status was negatively associated with blood pressure and plasma lipids (Petersen et al., 2015; Williams et al., 2012).

Fetal life may be a critical period for the effects of vitamin D deficiency because of the increased need and rapid fetal development. We have previously shown an association of maternal vitamin D with fetal growth patterns and birth outcomes (Miliku et al., 2016). For the current study, we hypothesized that suboptimal fetal vitamin D concentrations may increase the risk of adiposity and cardiovascular factors in the offspring. Results from previous birth cohort studies suggest a possible programming effect of maternal 25(OH)D concentrations on childhood body composition (Crozier et al., 2012; Krishnaveni et al., 2011). In a study among 977 women and their offspring, it was observed that lower maternal 25(OH)D concentrations during pregnancy were associated with higher fat mass in 6-year-old children (Crozier et al., 2012). Another study among 568 Indian women and their children reported that low maternal 25(OH)D concentrations were associated with a lower lean mass

in the offspring (Krishnaveni et al., 2011). In line with these findings, our results suggest that low maternal 25(OH)D concentrations during midpregnancy are associated with a higher fat mass percentage and fat mass index and lower lean mass percentage in the offspring. We observed that the associations of maternal 25(OH)D concentrations during pregnancy with childhood fat and lean mass percentage were independent of birth weight and childhood 25(OH)D concentrations. We did not observe any associations of cord blood 25(OH)D concentrations with childhood body composition. Furthermore, we observed tendencies for similar associations when we restricted the analyses to Europeans only. However, the associations were not significant, probably due to smaller numbers. It has been previously shown that body composition phenotypes track and are associated with poorer outcomes in later life (Hall, Crook, Jones, Wofford, & Dubbert, 2002; Vogelezang et al., 2016). Therefore, the observed subclinical differences in body composition in childhood may be related to adverse outcomes in later life.

Only few studies have explored the association of fetal 25(OH)D concentrations with childhood cardiovascular risk factors (Gale et al., 2008; Krishnaveni et al., 2011; Williams et al., 2013). In a recent study among 4,109 mothers and children at the ages of 9.9 and 15.4 years, a weak inverse association was observed of maternal 25(OH)D concentrations with systolic blood pressure at the age of 9 years (Williams et al., 2013). However, this association was not present at the age of 15.4 years (Williams et al., 2013). We did not observe any association between maternal 25(OH)D concentrations and blood pressure in 6-year-old children. Also, we did not observe any association of maternal or cord blood 25(OH)D concentrations with childhood total cholesterol, triglycerides, and insulin concentrations. A small study in India observed that children of vitamin D deficient mothers had higher insulin concentrations (Krishnaveni et al., 2011). It may be that the different results of our findings as compared with this previous study are explained by ethnic differences. When we restricted the analyses to Europeans only, we observed similar results as in the full group. Unfortunately, we did not have enough numbers in the other ethnic subgroups to perform ethnic specific analyses. Our results do not support in utero effects of 25(OH)D concentrations on fetal cardiovascular risk factors.

The mechanisms by which maternal 25(OH)D concentrations during pregnancy may affect offspring body composition are poorly understood. Animal experiments relate maternal 25(OH)D concentrations to fetal muscle development. A study in rats which tracked ^{3}H-labelled vitamin D injected into pregnant rats showed that 25(OH)D was transferred to the fetus and stored in fetal muscle tissue (Clements & Fraser, 1988). 1,25-Dihydroxycholecalciferol regulates adipocyte 11β-hydroxysteroid dehydrogenase Type 1 activity, which generates active cortisol from inactive cortisone. The expression and cortisol production in human adipocytes suggests a potential role for (1,25-dihydroxycholecalciferol) in fat mass (Morris & Zemel, 2005). Furthermore, vitamin D can inhibit the expression of a key adipogenesis regulator, peroxisome proliferator-activated receptor-gamma (Kong & Li, 2006; Wood, 2008).

Although the observed effect estimates are small and without direct individual clinical consequence, the results of this study suggest that maternal 25(OH)D concentrations are associated with offspring fat mass percentage and lean mass percentage. Suboptimal vitamin D levels in pregnant women may have persistent effects on their offspring body composition. An adverse childhood body fat profile may increase the risk of cardiovascular and metabolic diseases in later life. With our study design, it is not possible to establish the causality of these associations and further studies are required. However, our findings suggest that vitamin D supplementations during pregnancy may need to be targeted at women who are vitamin D severely deficient. In the Netherlands, pregnant women are advised to use vitamin D supplements (10 μg/day). Therefore, our results support population-strategies to improve vitamin D concentrations in pregnant women to optimize offspring growth and development.

4.2 | Strengths and limitations

A major strength of our study is the prospective design from fetal life onwards within a population-based cohort. This study is among the largest that examined the association of fetal vitamin D status with body composition and cardiovascular outcomes in a multi-ethnic sample of school-age children. We measured 25(OH)D concentrations in midpregnancy and cord blood at birth, assessing different critical periods. 25(OH)D concentrations are the most widely used indicator of vitamin D status (Harvey et al., 2014). Next to BMI, we also measured fat mass and lean mass using DXA. Due to the young age of the children, it was not possible to obtain fasting blood samples, which may have led to an underestimation of the associations. Of the singleton live born children 58% participated in the follow-up measurements. Mothers of the children who were lost to follow-up had on average lower 25(OH)D concentrations and were on average lower educated, suggesting that our study population had a selection towards a healthier population. A limitation of this study is that we did not have information on maternal vitamin D supplement use during pregnancy and childhood dietary intake of vitamin D or supplement use, or other food patterns. However, our group has previously shown that vitamin D intake and supplement use in early childhood were not associated with 25(OH)D concentrations at the age of 6 years (Voortman et al., 2015) Finally, although we performed adjustment for many potential maternal and childhood confounders, residual confounding for the observed associations might be present.

5 | CONCLUSION

Results from this population-based prospective cohort study suggest that severe vitamin D deficiency (<25 nmol/L) during midpregnancy may influence childhood body composition. Further studies are needed to examine the causality of these associations and to identify the long-term clinical consequences.

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CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

CONTRIBUTIONS

KM and VWVJ designed the research and wrote the paper; VWVJ was involved in the design and planning of the study and data collection; DE, TB, and JM performed the Vitamin D assays on maternal and cord blood. KM analysed the data; JFF, TV, HT, DE, TB, and JM provided comments and consultation regarding the analyses and manuscript; KM and VWVJ have primary responsibility for final content. All authors critically reviewed and gave final approval of the version to be published.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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